Kidney International, Vol. 47 (1995), pp. 299–305

TECHNICAL NOTE

Growth of cultured rabbit renal tubular cells does not require exogenous glutamine

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Following the pioneering work of Eagle et al [1], glutamine, which is the most abundant amino acid in plasma (0.5 to 0.6 mM) [2] and an important source of energy and nitrogen for cell metabolism and growth [3], is considered to be a mandatory component of cell culture media. In renal epithelial cell cultures, it is added often at a final concentration of 2 to 5 mM [4–10]. However, this is not necessarily useful in primary cultures of renal proximal tubular cells from the rabbit, the most widely used species for renal cell cultures. Indeed, glutamine synthetase is present in the renal cortex of this species; in addition, the activity of phosphate-activated glutaminase, the enzyme which initiates glutamine metabolism and yields glutamate and ammonium [3], is low in the rabbit kidney [2]. Moreover, freshly isolated rabbit proximal tubules do not or hardly remove glutamine in net amounts when they are incubated in the presence of glutamine concentrations varying from 1 to 5 mM [11].

In an attempt to clarify this point, we cultured rabbit proximal tubular cells on a permeable collagen membrane and investigated whether the addition of glutamine to the culture medium influences cellular growth and glycolysis.

The results obtained show that omission of exogenous glutamine does not alter the growth of rabbit renal proximal tubular cells in primary culture, and that the provision of glutamine by the glutamine synthetase reaction is sufficient and mandatory for their growth and DNA synthesis. Addition of glutamine, which stimulated glycolysis in freshly isolated proximal tubules, failed to do so in cultured rabbit renal cells.

Methods

Animals

New Zealand rabbits, 1.2 to 1.5 kg, (Elevage Scientifique des Dombes, Châtillon-sur-Chalaronne, France), were used for all studies, and were killed by an i.v. injection of sodium pentobarbital (5 ml of a solution of 0.03 g/ml).

Reagents

Glutaminase (grade V), L-glutamine, methionine sulfoximine, crystal violet and H 33258 dye were from Sigma Chemical Co. (St. Louis, Missouri, USA). Other enzymes and coenzymes were purchased from Boehringer (Meylan, France). Other chemicals were obtained from Merck (Darmstadt, R.F.A.). Medium for cell cultures was purchased from Eurobio (Les Ulis, France).

Freshly isolated kidney tubules

Preparation of kidney cortex tubules. The kidney cortices of one rabbit were sliced with a hand-microtome and the renal tubules were isolated by collagenase digestion as previously described by Baverel et al [12]. The tissue suspension obtained was used immediately after preparation.

Incubation procedures. Incubations were carried out at 37°C in a shaking water bath, in 25 ml Erlenmeyer flasks, in an atmosphere of 95% O_2-5% CO_2. Tubules were incubated for 60 minutes in 4 ml of Krebs-Henseleit buffer [13] with 5 mM glucose and in the absence or the presence of glutamine (from 0.5 to 8 mM) or of NH_4Cl (from 0.25 to 10 mM).

All experimental conditions were carried out in duplicate. Incubations were terminated by adding $HClO_4$ (final concentration 2% vol/vol) to each flask. In all experiments, zero-time flasks with and without substrates, were prepared by adding $HClO_4$ before the tubules. After removal of the denaturated protein by centrifugation (4,000 g for 10 min), the supernatant was neutralized with a mixture of KOH (20%; wt/vol)/H_3PO_4 (0.15 M), (99:1; vol/vol) before metabolites were assayed.

Cell cultures

Culture media and supports. The preparation medium was a mixture of DMEM (Dubelcco's modified Eagle's medium)/HAM F12 (50:50), pH 7.4, containing $NaHCO_3$ (25 mM), Hepes (10 mM), penicillin (100 U/ml), streptomycin (100 mg/ml), and glucose (1 g/liter), without glutamine.

The complete medium used for the seeding contained in addition fetal calf serum (10%), insulin (5 μg/ml), EGF (10 ng/ml), triiodothyronine (1.10^{-8} M), hydrocortisone (5.10^{-8} M), sodium selenite (3.10^{-8} M), and transferrin (5 μg/ml). After three days, the culture medium was deprived of antibiotics and fetal calf serum. When necessary, glutamine or ammonium chloride were introduced into the medium just before use in order to avoid the uncontrolled degradation of glutamine occurring during storage.

The preparation media were equilibrated with 95% O_2-5% CO_2.

Cells were cultured on pure collagen permeable membranes mounted on 12 mm inserts for 12 well plates and made in our

Received for publication February 14, 1994
and in revised form August 8, 1994
Accepted for publication August 12, 1994

Table 1. Effect of glutamine on glucose metabolism in rabbit kidney cortical tubules

Experimental condition	ATP content *μmol/g dry wt*	Metabolite removal (−) or production *μmol/g dry wt*				
		Glucose	Lactate	Glutamine	Glutamate	NH_4^+
Glucose (5 mM)	7.1 ± 0.4	−105.4 ± 8.8	62.5 ± 17.3	18.7 ± 2.3	11.0 ± 1.7	0.1 ± 3.2
Glucose (5 mM) + glutamine (0.5 mM)	7.1 ± 0.4	−111.6 ± 10.4	67.8 ± 20.6	22.7 ± 3.7	12.5 ± 1.6	−4.6 ± 1.2
Glucose (5 mM) + glutamine (1 mM)	7.1 ± 0.3	−121.0 ± 11.1	77.6[a] ± 21.4	19.2 ± 4.7	13.5 ± 1.9	−1.6 ± 1.0
Glucose (5 mM) + glutamine (2 mM)	6.6 ± 0.4	−125.5[a] ± 12.1	80.5[a] ± 20.8	22.0 ± 5.9	20.4[a] ± 2.3	−2.0 ± 1.1
Glucose (5 mM) + glutamine (4 mM)	7.1 ± 0.4	−144.9[c] ± 13.6	104.2[a] ± 25.7	27.2[a] ± 3.4	25.5[b] ± 2.6	5.3 ± 1.4
Glucose (5 mM) + glutamine (8 mM)	7.1 ± 0.3	−168.5[c] ± 9.7	119.0[b] ± 24.0	−25.8[b] ± 7.3	29.7[a] ± 2.9	31.2[a] ± 3.7
No added substrate	6.0 ± 0.3	−0.2 ± 0.2	−4.2 ± 0.3	41.4 ± 6.4	−3.0 ± 0.9	5.4 ± 2.8

Freshly isolated kidney tubules (49.2 ± 4.3 mg dry wt per flask) were incubated for 60 min as described in the **Methods** section, in the presence of 5 mM glucose and increasing concentrations of glutamine. Glucose, lactate, ATP, glutamine, glutamate and NH_4^+ were determined as described in the **Methods** section. Net substrate utilization and product formation were calculated as the difference between the total content (tissue + medium) of the incubated flask and the zero-time flask. Results, in μmol of substance removed or produced per g dry wt of tubules per hr, are expresssed as means ± SEM for four experiments performed in duplicate. Statistical difference was measured by the paired Student's *t*-test against the control without glutamine.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$

laboratory as follows: a nylon net of 1 mm mesh size was clipped onto the bottom of a plastic cylinder as a drum membrane, laying 1 mm above the bottom of the multiwell plate by means of three feet. Under the hood, the nylon net was soaked in rat tail acid soluble collagen I (3 mg/ml in 0.1% acetic acid) [14], and the liquid film remaining inside the meshes dried in 3% ammonia vapors during 15 minutes, then one hour under the hood. The dry collagen mounted inserts were stored for weeks at room temperature.

Cell preparation and culture. Isolated proximal tubular cells were prepared according to Poujeol and Vandewalle [15]. The cells ($1.125\ 10^6$ cells/cm^2), were seeded into the inserts in 0.5 ml of complete medium; finally, 1.75 ml complete medium was added in the well outside the insert. Some inserts were incubated without cells as control for protein and metabolite determinations.

Culture plates were maintained at 37°C in a water-saturated atmosphere of 5% CO_2-95% air. The culture medium (complete medium without antibiotics and calf serum) was replaced three days after seeding and then every day.

On the fourth and seventh day, samples of medium (inside and outside of the insert) were taken in order to assess the metabolite removal or production during 24 hours. These samples were deproteinized by adding $HClO_4$ (final concentration 2% vol/vol) and stored at −18°C. After neutralization with a mixture of KOH (20%; wt/vol)/H_3PO_4 (0.15 M), (99:1; vol/vol), the metabolites were assayed.

Analytical methods

Cellular aminopeptidase M activity was determined *in situ* with L-alanine-nitro-4-anilide as substrate by the method of George and Kenny [16] as follows: after rinsing the cells with phosphate buffered saline (PBS), the reagent (L-alanine-nitro-4-anilide, 1.5 mM, in sodium phosphate buffer, 50 mM, pH 7) was added inside and outside the insert (500 μl and 1500 μl, respectively) and incubated at 37°C during 20 minutes. Incubations were terminated by pipetting the total volume out of the well and adding 0.1 ml of 10% (wt/vol) sodium dodecyl sulfate. The optical density was read at 410 nm with p-nitroaniline as reference.

Cellular protein and DNA contents were determined in the same cells after solubilization with the alkaline solution A+B+C of Lowry et al [17], which did not spoil the collagen film (500 μl and 1500 μl inside and outside the insert, respectively) during 30 minutes at room temperature. Then, the total volume of the well was pipetted out. Proteins were determined with the reagent of Bradford [18], which doesn't react with collagen, bovine serum albumin being used as reference. After neutralization to pH 7.2 to 7.5 of Lowry's reagent with citric acid (0.4 M), DNA was determined fluorometrically with H 33258 dye by the method of Labarca and Paigen [19]. Single stranded DNA was used as reference since control experiments indicated that Lowry's reagent is a denaturating agent.

Cell counts. The cellular membranes together with the collagen film were disrupted with 2 ml citric acid (0.1 M) during 15 minutes at room temperature. The resulting nuclei suspension was then stained with crystal violet and counted [20].

Glucose, lactate, ammonium, glutamate, glutamine and ATP were determined enzymatically with a spectrophotometric method recording changes in the concentration of NADH or NADPH [21–26].

Resistance of the cell monolayer was determined with a MILLICELL®-ERS ohmmeter.

Electron microscopy was performed by the Service Commun de Microscopie Electronique (UFR médicale A. Carrel, Lyon, France).

Results

Freshly isolated rabbit kidney tubules

When rabbit kidney tubules, whose viability was indicated by a high cellular ATP content, were incubated with both 5 mM glucose and various concentrations of glutamine, glucose was removed under all experimental conditions. In contrast, net removal of

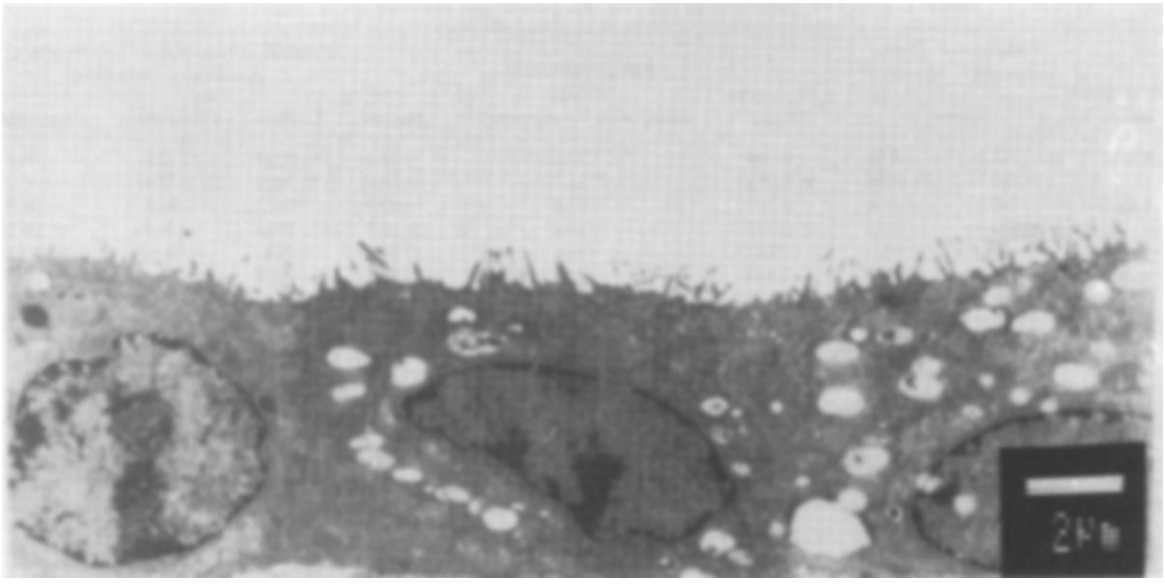

Fig. 1. *Cultured rabbit renal proximal cells 7 days after seeding.* Transmission electron microscopy view showing a regular monolayer with tall cells, numerous intercellular digitations, and a well-developed brush border.

glutamine occurred only at the highest concentration (8 mM) of glutamine used (Table 1) and at low rates (under this condition, 41% of the glucose and 4% of the glutamine present at zero time were removed). Under all other experimental conditions, a small net production of glutamine was observed (Table 1). With 8 mM glutamine as substrate, the accumulation of NH_4^+ suggests that glutamine was metabolized by glutaminase which yielded glutamate and NH_4^+.

Table 1 also shows that, at concentrations from 2 to 8 mM, glutamine caused a significant dose-dependent increase in glucose utilization. In the presence of 4 mM glutamine, a concentration often found in culture media, a 38% increase was observed. The stimulation of glucose utilization was accompanied by an increase in lactate accumulation which was also observed in the presence of 1 mM glutamine (Table 1).

Since NH_4^+ accumulated in the presence of the highest concentrations of glutamine used, which caused the most pronounced stimulation of glycolysis (Table 1), we tested whether the glutamine effects were mediated, at least in part, by NH_4^+. When tubules were incubated with 5 mM glucose in the presence of 0.25, 0.5, 1, 5 and 10 mM of NH_4Cl, glucose removal was indeed stimulated by 19, 28, 42, 111 and 147%, respectively. Under these conditions, lactate accumulation was stimulated by 17, 31, 51, 138 and 191%, respectively (results not shown).

Primary culture of rabbit renal proximal tubular cells

The perfect transparency of our collagen film allowed a good observation of cellular growth, and provided an excellent surface for cell anchorage and division. The cells reached confluency within four to five days. After seven days, the monolayer exhibited a regular array of tall cells with well-developed microvilli of about 1 μm height (Fig. 1).

The protein and DNA content doubled between day 4 and day 7 (Table 2); after seven days of culture, the cell monolayer contained a mean of 182 μg protein/cm^2, a value well above that of cells cultured on a dish bottom which usually ranges from 15 to 50 μg/cm^2 [4, 5, 27, 28]. The protein/DNA ratio was twice that of freshly isolated cells (79.9 ± 8.7 and 38.3 ± 4.7, respectively).

The activity of alanine aminopeptidase, a marker of apical brush border, which was equal to 141 ± 35 nmol/min/mg protein (N = 6) in freshly isolated proximal tubules, decreased to 25 ± 3 nmol/min/mg protein in cultured cells at day 7 (Table 2). A similar decrease in aminopeptidase activity was also found by other authors in cultured cells at day 6 when compared to that in freshly isolated tubules [4–6].

After confluency, the cell monolayer exhibited an electrical resistance averaging 22 $\Omega \cdot cm^2$ on day 7 (N = 13). This low resistance, which is in the same range as that measured by Bello-Reuss and Weber [7] and very different from that measured on distal tubules and collecting ducts (100 to 1000 $\Omega \cdot cm^2$), is typical of a leaky epithelium such as the proximal tubule [29].

After confluency, the cell monolayer became tight enough to establish a glucose gradient: at day 4 there was no glucose gradient, whereas at day 7 a basolateral/apical glucose gradient of 8.6 ± 3.8 (N = 5) was found.

Table 2 shows that, both at day 4 and day 7, our cultured cells were highly glycolytic since glucose removal per mg protein (assuming that 85 to 90% of the tubular dry weight is represented by protein) was about 20 times higher than that in freshly isolated kidney tubules (Table 1). At day 7, about 70% of each well glucose content was consumed by the cell monolayer within 24 hours. Given that one glucose molecule can yield two lactate molecules, 80 to 90% of the glucose removed was converted into lactate (Table 2), whereas in freshly isolated tubules, the percentage of the glucose removed giving rise to lactate was much lower (Table 1).

Effects of glutamine and inhibition of glutamine synthetase. Table 2 shows that none of the three growth parameters (that is, protein and DNA content and cell counts) was modified by glutamine addition to the medium, neither at day 4 nor at day 7.

In contrast with what was observed in freshly isolated tubules, addition of 4 mM glutamine failed to stimulate glucose removal

Table 2. Effect of glutamine (4 mM) on the growth and metabolism of cultured cells

Age of culture	Glutamine added	Protein content	DNA content	Cell counts	Aminopeptidase activity	Metabolite removal (−) or production *μmol/mg protein/24 hr*				
		μg/cm²		$10^6/cm^2$	*nmol · min⁻¹ · mg prot⁻¹*	Glucose	Lactate	Glutamate	Glutamine	NH_4^+
Day 4	none	82.1	1.117	0.361	39.2	−53.5	93.6	2.1	0.8	1.2
		± 16.7	± 0.28	± 0.087	± 11.3	± 18.0	± 36.0	± 0.8	± 0.3	± 0.7
	4 mM	88.3	1.161	0.363	63.0	−60.9	96.0	40.9	−60.5	37.6
		± 20.7	± 0.23	± 0.098	± 10.0	± 19.5	± 24.0	± 8.9	± 19.1	± 10.8
P		NS	NS	NS	NS	NS	NS	< 0.02	< 0.05	< 0.05
Day 7	none	182.0	2.454	0.894	24.7	−52.2	92.8	0.3	0.9	−1.0
		± 19.6	± 0.141	± 0.105	± 2.7	± 6.7	± 13.6	± 0.1	± 0.7	± 0.6
	4 mM	180.6	2.279	0.840	25.9	−45.9	77.9	2.3	−3.2	2.3
		± 18.8	± 0.158	± 0.080	± 1.9	± 4.2	± 8.3	± 0.3	± 1.3	± 1.0
P		NS	NS	NS	NS	NS	NS	< 0.01	< 0.02	< 0.05

Cells were cultured as described in the **Methods** section with 5 mM glucose, in the absence or the presence of 4 mM glutamine in the medium. Protein and DNA content, cell number, aminopeptidase activity, glutamine and glucose removal, and the accumulation of lactate, glutamate and NH_4^+ were determined on day 4 or day 7. Results are expressed as means ± SEM for 5 experiments with each point in triplicate. Substrate utilization and product formation were calculated as the difference between metabolite content of medium (inside + outside) in the control wells without cells and the wells containing cells. Statistical difference was measured by the paired Student's *t*-test against the control without glutamine; NS is not significant.

and lactate accumulation at days 4 and 7. Neither at day 4 nor at day 7 was the specific activity of alanine aminopeptidase significantly modified by the presence of glutamine (Table 2).

Table 2 also shows that glutamine was removed by the cultured cells at relatively high rates at day 4 (during the growth phase) but not at day 7 (after confluency). At day 4, glutamine removal was associated with a large increase in the accumulation of both glutamate and NH_4^+ which accounted for about two-thirds of the glutamine removed.

The observation that growing cells utilize exogenous glutamine, though an exogenous supply of this amino acid is not required, suggested that glutamine is needed for such a growth and led us to investigate the potential importance of glutamine synthesis for cell growth. For this, we conducted experiments in which we used methionine sulfoximine (MSO), a potent inhibitor of glutamine synthetase [3]. The results of these experiments are shown in Table 3. When 1 mM MSO was introduced into the culture medium from day 0, the cells were able to anchor and spread, but no growth was observed; thereafter, the seeded cells died and became detached from the collagen support. When the culture medium containing MSO was supplemented with 4 mM glutamine, the cellular growth was fully restored, indicating that inhibition of glutamine synthetase was responsible for the cytotoxic effect observed upon addition of MSO alone. When MSO was introduced into the culture medium only from day 4, the growth was stopped and the protein content at day 7 was lower than that found at day 4 for cells cultured without MSO (Tables 2 and 3).

Effect of NH_4Cl. In contrast with what was observed in freshly isolated tubules, the presence of either 2 or 10 mM NH_4Cl failed to stimulate glucose removal when measured at day 4; similar results were obtained at day 7 when 2 mM NH_4Cl was present from day 0. On the contrary, the presence of 10 mM NH_4Cl from day 0 led to a 58% and 72% inhibition of glucose removal and lactate accumulation, respectively. However, addition of 2 mM NH_4Cl from day 4 led to a 22% stimulation of lactate accumulation (results not shown).

Table 3. Effect of methionine sulfoximine on the growth of cultured cells

Culture condition	Cell counts $10^6/cm^2$	Protein content *mg/cm²*	DNA/protein μg/mg
Control without GLN	0.894	0.182	14.1
nor MSO	± 0.105	± 0.020	± 1.6
MSO (1 mM)	0.075	0.018	6.0
added from day 0	± 0.024	± 0.004	± 1.5
P	< 0.01	< 0.001	< 0.01
MSO (1 mM) + GLN (4 mM)	0.854	0.173	12.8
added from day 0	± 0.048	± 0.019	± 1.9
P	NS	NS	<0.05
MSO (1 mM)	0.279	0.061	11.2
added from day 4	± 0.103	± 0.017	± 1.5
P	< 0.001	< 0.001	< 0.05
MSO (1 mM) + GLN (4 mM)	0.830	0.176	10.8
added from day 4	± 0.122	± 0.017	± 1.3
P	NS	NS	< 0.05

Cells were cultured as described in the **Methods** section. Methionine sulfoximine (MSO), a potent inhibitor of glutamine synthetase, was added in the medium either from day 0 or from day 4, either in the absence or the presence of 4 mM glutamine (GLN). Cell counts and protein and DNA determination were performed 7 days after seeding. Results are expressed as means ± SEM for five experiments (each point in triplicate). Statistical difference was measured by the paired Student's *t*-test against the control without glutamine; NS is not significant.

Discussion

Our cultured cells retained features characteristic of proximal tubules, as indicated by several lines of evidence: (i) morphologically, they exhibit an apical brush border membrane with numerous microvilli (Fig. 1), (ii) biochemically, they contain a high activity of alanine aminopeptidase, a well-established brush border marker enzyme, (iii) their electrical resistance at confluency is low and in the same range as that demonstrated in proximal tubules and greatly differs from that shown in distal nephron segments [29]; and (iv) they are able to establish an apical-to-basolateral glucose gradient which is specific to the proximal tubule [30].

Lack of requirement of exogenous glutamine for the growth of rabbit renal proximal tubular cells in primary culture

A new finding of the present work is that addition of glutamine to the culture medium, which is usual in studies performed with

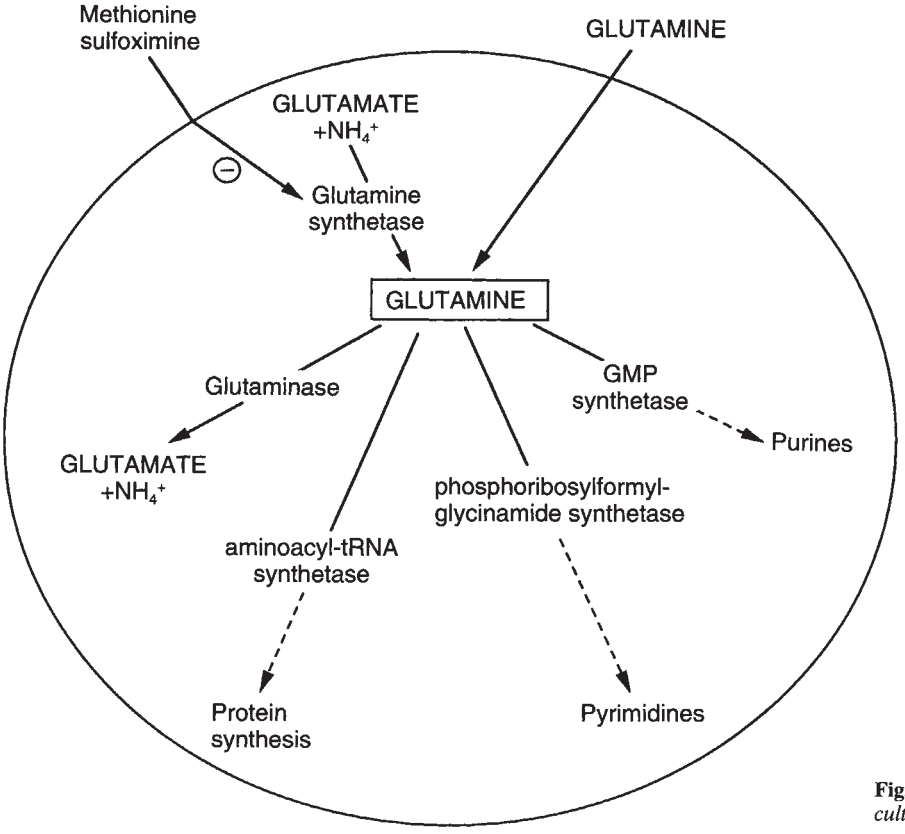

Fig. 2. *Pathways of glutamine metabolism in cultured rabbit proximal tubular cells.*

rabbit proximal tubular cells in primary culture [4–10], is not necessary for the growth of these cells (Table 2). However, this does not mean that glutamine, which provides the nitrogen needed for purine and pyrimidine nucleotide biosynthesis [3, 31], was not required for the growth of our cells. That, in the absence of exogenous glutamine, the needed glutamine was provided by the operation of glutamine synthetase, is demonstrated by the absence of cellular growth and even by cell death when glutamine synthetase was inhibited by methionine sulfoximine (Table 3). This observation indicates that rabbit proximal tubular cells in primary culture retain enough glutamine synthetase activity, which is high in parent cells [2], to cope with their own glutamine requirements. In addition, the presence of glutamine synthetase in our cultured cells provides additional evidence that they expressed proximal tubular functions because this enzyme activity is exclusively localized in the intact rabbit proximal tubule [32].

Interestingly, our results show that, during proliferation but not after confluency, rabbit proximal tubular cells in primary culture can remove exogenous glutamine (Table 2). This highlights the role of glutamine as precursor of cellular constituents during cellular growth [3, 31]. It should be pointed out here that a key role of glutamine in cell growth and survival seems to be the supply of nitrogen for nucleic acid synthesis since, as shown by the DNA/protein ratio in Table 3, in the absence of glutamine availability (exogenous and endogenous), it is the DNA content which is the most affected. However, most (about two-thirds) of the glutamine removed could be accounted for by glutamate and ammonium accumulation (Table 2), which strongly suggests that most of glutamine utilization occurred by the operation of the glutaminase reaction which is also present in parent cells [2]. The fact that stoichiometric amounts of glutamate and ammonium accumulated when glutamine was removed also indicates that the glutamate dehydrogenase pathway was not functional. This observation together with the low rate of glutamine utilization and the limitation or absence of its metabolism beyond the stage of glutamate strongly suggest that, under our experimental conditions, glutamine carbon could not be oxidized to a significant extent and, therefore, could not represent a significant source of energy as shown for other cultured cell types like Hela cells [1]. However, whether this would also hold in the absence of glucose is uncertain.

Glutamine addition and dedifferentiation of rabbit proximal tubular cells in primary culture

Dedifferentiation of cultured cells represents a severe pitfall for their utilization as experimental models [33]. For example, the activity of alanine aminopeptidase, a brush border membrane enzyme, is decreased in cultured renal cells when compared with that in freshly isolated cells [4–6]. In addition, renal proximal tubular cells, which are gluconeogenic when they are freshly isolated [34], lose their gluconeogenic capacity and become highly glycolytic in culture [5, 7, 35, 36]. It has been shown that this dedifferentiation is modulated by the presence of substrates and growth factors in the culture medium [37–39].

Our data confirm the previous observation that rabbit proximal tubular cells in primary culture exhibit a high rate of glycolysis (Table 2). However, the view that the metabolism of cultured

proximal cells reverts from gluconeogenesis to glycolysis [8, 40] should be amended at least for rabbit proximal tubular cells because freshly isolated rabbit proximal tubules have the capacity, even though much lower than that of cultured cells, of metabolizing glucose (Tables 1 and 2).

In our experiments, glutamine addition to the medium did not prevent the increase in the glycolytic capacity nor the decrease in alanine aminopeptidase activity observed when isolated cells are cultured. This differs from the results obtained by Blais et al [28], who demonstrated that glutamine addition to the culture medium prevented the loss of brush border enzyme activities. However, it should be noted that Blais et al [28] added glutamine to the culture medium and simultaneously removed glucose whereas we added glutamine without removing glucose; the fact that the omission of glucose partially prevents the dedifferentiation of rabbit proximal tubular cells in primary culture [8] might explain the preventive effects attributed to glutamine by Blais et al [28]. In addition, it should be underlined that, unlike ours, the cells used by Blais et al [28] were cultured in the absence of EGF and triiodothyronine, two physiological hormones that play a role in renal growth [41, 42]; the lesser dedifferentiation of cells cultured in such a medium is obtained at the cost of a slower growth, as reflected by their protein content per cm^2 and brush border enzyme activity, respectively [28] (Table 2).

It should also be stressed that the high rate of glycolysis observed in our cultured cells is not due to an activating effect of glutamine or of NH_4^+, as could have been expected from experiments with freshly isolated tubules (Table 1 and **Results**). Such a loss of activating effects by these two compounds suggests that, in cultured cells, the target of the glutamine and NH_4^+ effects in freshly isolated cells, which is possibly phosphofructokinase [43], has been modified or was already fully activated.

Conclusion

This study demonstrates that, although rabbit proximal tubular cells in primary culture have an absolute requirement of glutamine for their growth, the addition of glutamine to their culture medium is not necessary and therefore can be avoided. This might also be the case for primary cultures of rat renal proximal tubular cells whose parent cells contain glutamine synthetase activity [2]. In contrast, primary cultures of human and dog renal proximal tubular cells, which contain no glutamine synthetase activity *in situ* [2], are expected to need exogenous glutamine for their cellular biosyntheses. These results illustrate once again that each cell type has special requirements for both proliferation and expression of specific functions. They also suggest that definition of the proper medium for sustaining growth and differentiation should take into account the biochemical and functional properties of the parent cells *in vivo*.

Acknowledgments

This work was supported by the C.N.R.S. (E.P. 18) and by grants from the Ministère de la Recherche et de la Technologie (n°91G 0248), the INSERM, Paris, France (CRE n° 900708) and the Département de Biologie Humaine, Lyon, France. Thanks to Philippe Poujeol and his coworkers (Saclay, France) for kind technical initiation and helpful discussions.

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